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Bacterial Cell Length and Motility Measurement

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An abbreviated version of this protocol was published in eLIFE in Apr 2021

Confinement discerns swarmers from planktonic bacteria

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Detailed protocol

Protocol: Bacterial Cell Length and Motility Measurement

For planktonic SM3 (always use a strain whose purity was confirmed previously),

1. Dilute 50 µL SM3 overnight culture (~ 16 h) in 5 mL autoclaved LB (Lysogenic Broth: 1 g tryptone, 0.5 g yeast extract, 0.5 g NaCl, 100 mL deionized water) and incubate the culture in a 37°C shaker (200 rpm) for 2.5 h.
2. Dilute the regrown culture 1:100 in autoclaved LB.
3. After gentle mixing, use a pipette to transfer a droplet of 50 µL diluted bacterial culture onto a glass slide.
4. Cover the droplet of bacterial culture with a cover slip (Fisherbrand, Cat. No. 12544DP) and place the slide on the microscope stage for observation using a 20x objective.
5. Open the microscope camera software (Camera: Kiralux CS505MU, Thorlabs; Software: ThorCam Software, Thorlabs), and adjust the focus nob so that bacterial cells can be seen clearly in the live view.
6. Record a sequence of images of the bacterial motion at 10 fps (frames per second) for 5 to 10 sec.
7. Open the image sequence in ImageJ (v.1.59e) and set the scale per the calibration of the microscope camera and the objective used (e.g., input “Known distance = 10 µm, Distance in pixels = 29” in the “set scale” user interface).
8. To measure the cell lengths, pick any one frame in the image sequence, use the “freehand tool” in ImageJ to label the cells respectively and then click “measure” to calculate the cell lengths in the unit of micrometer.
9. To measure the cell speed, record the coordinate (x, y) of one individual cell in one frame and then record the coordinate (x’, y’) of the same cell 10 frames afterwards, making sure that the cell’s trajectory is a straight line during the motion over the 10 image frames.
10. Calculate the traveling distance $d = [(x'-x)^2 + (y'-y)^2]^{1/2}$, and the speed of the particular cell is $v = d/t$. Here, $t \sim 1$ s since in our case the time interval spans 10 frames. (The frame rate is 10 fps, thus t = (10 frames)/ (10 frames/second) = 1 s).

For swarming SM3 (pre-testing of SM3 swarming on the strain at hand must be performed on agar to verify swarming conditions and ability),

1. Inoculate 2 µL overnight (~ 16 h) SM3 culture onto an LB agar plate (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, and 5 g/L agar in deionized water; volume = 20 mL/ 9 cm Petri dish). Place the plate in a 37°C incubator. When SM3 swarmed for ~ 2.5 h, use a spatula to collect a small chunk (~ 0.2 cm^2) of cells from the swarming colony edge and then transfer it into 1 mL autoclaved LB in a centrifuge tube.
2. Repeat step 3 – 10 to measure the cell length and speed of swarming SM3.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Chen, W. , Mani, S. and Tang, J. X.(2021). Bacterial Cell Length and Motility Measurement. Bio-protocol Preprint. bio-protocol.org/prep1104.
2. Chen, W., Mani, N., Karani, H., Li, H., Mani, S. and Tang, J. X.(2021). Confinement discerns swarmers from planktonic bacteria. eLIFE. DOI: 10.7554/eLife.64176